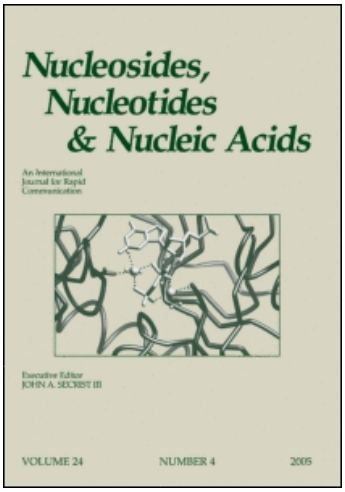

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Synthesis of Glucuronides of Fluoropyrimidine Drugs: N- and O-Glucuronides of 5-Fluorocytosine and 5-Fluorouracil

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SYNTHESIS OF GLUCURONIDES OF FLUOROPYRIMIDINE DRUGS: N- AND O-GLUCURONIDES OF 5-FLUOROCYTOSINE AND 5-FLUOROURACIL

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ABSTRACT: The protected (4a-9a) and deprotected (4b-6b, 8b) glucuronides of 5-fluorocytosine and 5-fluorouracil were synthesized and characterized by mass spectrometry and ^{19}F, ^{1}H and ^{13}C NMR. The substitution position of the sugar moiety on the pyrimidine ring was determined from the ^{13}C NMR chemical shift of the C1' of the sugar. The α or ß configuration of the glucuronide linkage was assigned on the basis of the value of the coupling constant between H1' and H2' of the sugar.

INTRODUCTION

The formation of glucuronides of two fluoropyrimidine drugs, (5-fluorouracil (FU) used in the treatment of solid tumors, and 5-fluorocytosine (FC) used in the treatment of severe fungal infections) has recently been reported[1,2]. In both cases, these covalent conjugates of ß-D-glucuronic acid were characterized by examination of the aglycon released by specific enzymatic hydrolysis using ß-glucuronidase. However, the position of the conjugation on the aglycon could not be assigned as FU and FC are multifunctional compounds. In an attempt to determine the exact structure of the glucuronide of FC found in the urine of patients treated with this drug[2], we characterized all the possible glucuronides of this drug by NMR (^{19}F, ^{1}H and ^{13}C) and mass spectrometry. Since we were not able to find any description in the literature of the preparation of these glucuronides, we developed their synthesis, which forms the subject of this report. Since the methods can also be applied to FU, we synthesized two glucuronides of FU and characterized their structures. The structures of the compounds synthesized are shown in FIG. 1.

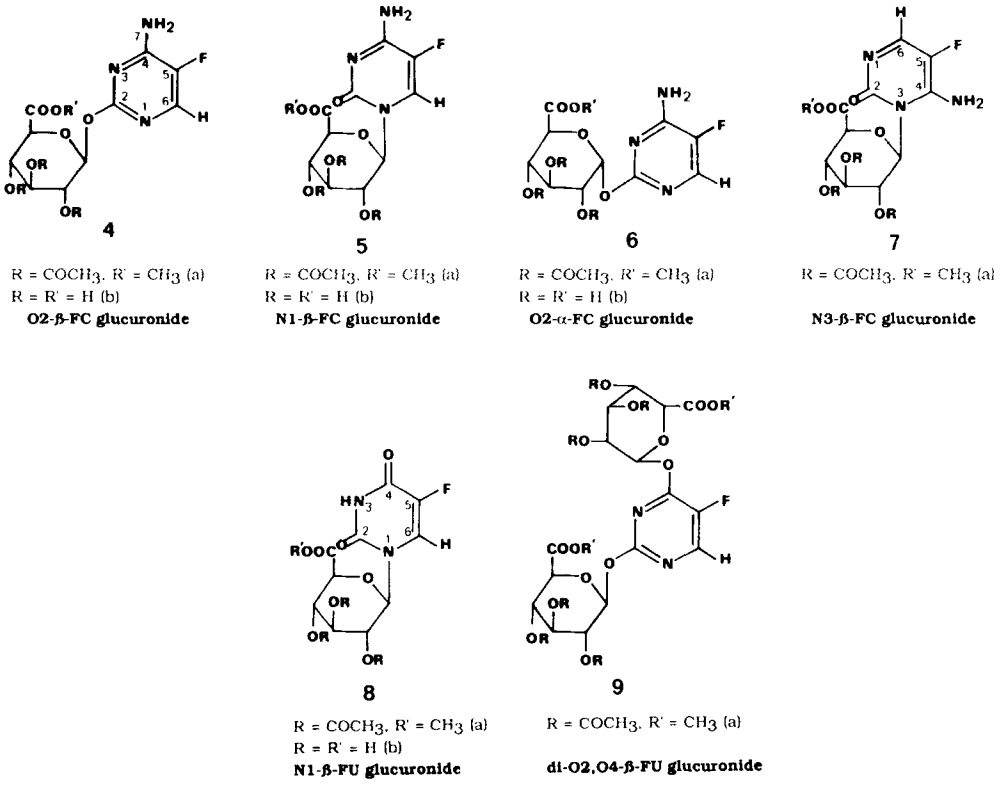


FIG. 1. Structures of the different glucuronides synthesized from 5-fluorocytosine and 5-fluorouracil.

RESULTS AND DISCUSSION

Synthesis of FC glucuronides

In common with cytosine, FC has four nucleophilic sites (N1, O2, N3 and N7), and so four monoglucuronides could be formed. From the studies of Ulbricht et al.[3-5] and Vorbruggen et al.[6-8] on the synthesis of pyrimidine glucosides, it appeared that the preferential synthesis of the O2-glucoside of cytosine was obtained by activating the carbonyl group of cytosine[3], and that of the N1-glucoside by protecting cytosine by silylation[6,8a]. So, we inferred that the condensation of cytosine itself with the sugar could afford a mixture of several glucosides. Using these methods, we were able to prepare four monoglucuronides of FC.

Specific synthesis of methyl 2,3,4-Tri-O-acetyl-1-(4-amino-5-fluoro-pyrimidin-2-yl)-ß-D-glucopyranosiduronate (protected O2-ß-FC glucuronide) 4a and 1-(4-amino-5-fluoro-pyrimidin-2-yl)-ß-D-glucopyranosiduronic acid (O2-ß-FC glucuronide) 4b

The protected glucuronide 4a was synthesized by condensation of the silver salt of FC with methyl 2,3,4-Tri-Q-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate 3, which was prepared in two steps from D-(+)-glucurono-3,6-lactone 1 (FIG. 2) according to the method described by Ulbricht and Rogers[3a].

The desorption chemical ionization (DCI) mass spectrum of 4a showed the parent ion $[MH]^+$ at m/z 446. Loss of the glucuronic acid moiety by glycosidic cleavage produces an ion at m/z 130 $(aglycon + H)^+$ characteristic of the protonated FC. The pyronium ion $(C_{13}H_{17}O_9)^+$ appears at m/z 317[9,10].

Analysis of the NMR spectra showed that compound 4a corresponded to the O2-ß-glucuronide of FC. The structure of the cytosine ring and the sugar was well characterized from the ^1H and ^{13}C NMR spectral data (TABLES 1,2). The ^{13}C NMR chemical shift of the C1' of the sugar (95.2 ppm) indicated that the aglycon and the glucuronic acid moiety were joined by a C1'-O linkage. The ß configuration of the glucuronide linkage was assigned on the basis of the large coupling constant between H1' and H2' ($J_{H1'-H2'}$ = 8.1 Hz), indicative of axial-axial coupling ($J_{H1'-H2'} \approx$ 8-10 Hz for the ß anomer versus ≈ 4 Hz for the α anomer)[10].

Careful removal of the protecting groups (acetylated and methylated) in sodiated medium afforded the O2-ß-glucuronide of FC 4b. The negative ion fast atom bombardment (FAB) mass spectral data showed the presence of molecular ions at m/z 304 $[M-H]^-$ and 326 $[M-H+Na]^-$ as expected since the compound was not purified from the sodiated medium.

The C1' resonance located at 99.6 ppm in the ^{13}C NMR spectrum (TABLE 4) is characteristic of a C1'-O linkage. In the ^1H NMR spectrum (TABLE 3), the anomeric proton (H1') resonance at 5.61 ppm exhibited a coupling constant $J_{H1'-H2'}$ = 6.8 Hz indicating a ß-glycosidic linkage.

Specific synthesis of methyl 2,3,4-Tri-O-acetyl-1-deoxy-1-(5-fluorocytosin-1-yl)-ß-D-glucopyranuronate (protected N1-ß-FC glucuronide) 5a and 1-deoxy-1-(5-fluorocytosin-1-yl)-ß-D-glucopyranuronic acid (N1- ß-FC glucuronide) 5b

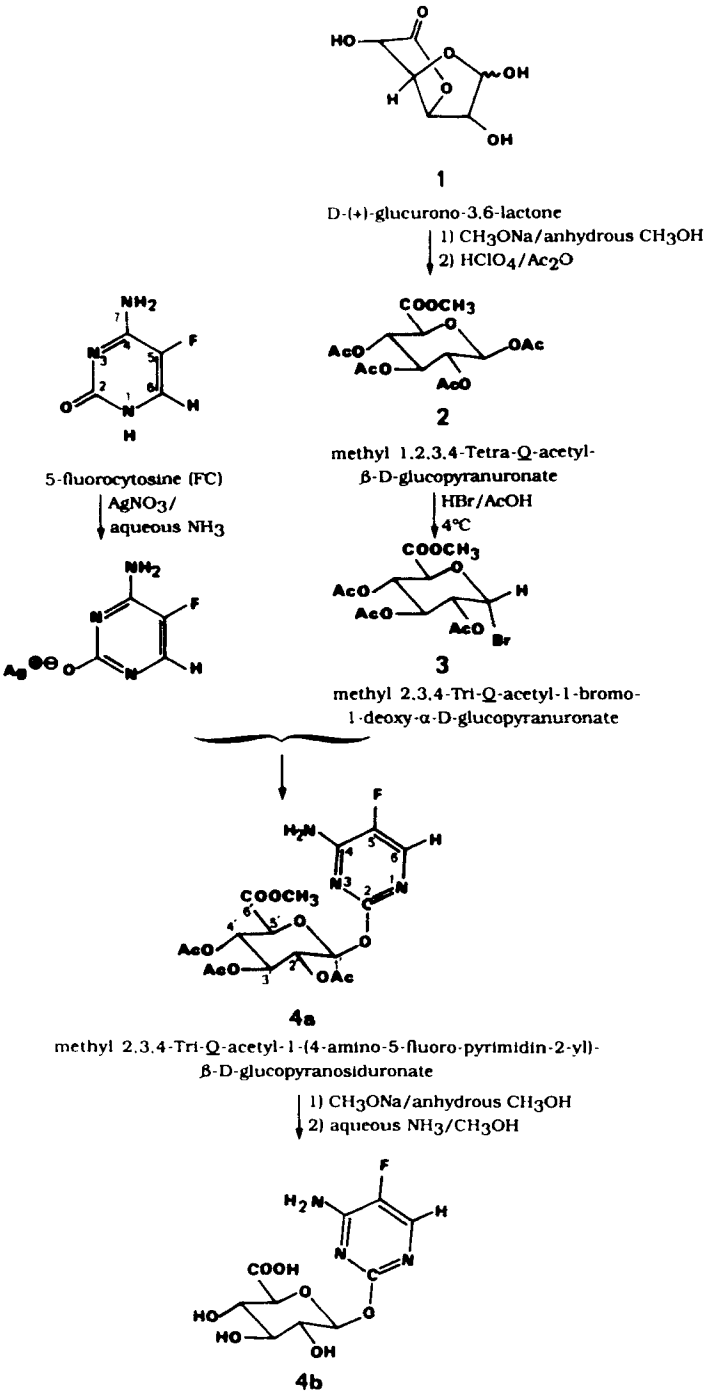


FIG. 2. Synthesis of protected and deprotected O2-ß-FC glucuronides 4a and 4b.

TABLE 1. ^{1}H NMR characteristics of protected glucuronides of FC and FU.

	∂, multiplicity, J[a]					
compound	O2-ß-FC 4a	O2-α-FC[b] 6a	N1-ß-FC[c] 5a	N3-ß-FC 7a	N1-ß-FU[c] 8a	di-O2,O4-ß-FU[d] 9a
H6	7.92, d, 3.0[e]	7.93, d, 3.0[e]	8.12, d, 7.0[e]	7.17, d, 5.0[e]	8.27, d, 6.9[e]	8.45, d, 2.4[e]
NH_2 or NH	6.77, s (b)[f]	6.82, s (b)[f]	7.15, s (b)[f]	7.83, s (b)[f]	10.6, s (b)[f]	
H1'	6.22, d, 8.1	6.60, dd, 3.6, 0.5	6.15, dd, 9.4, 2.1[e]	6.66, d, 9.4	6.10, dd, 9.3, 1.7[e]	6.49, d, 7.5 (A) 6.32, d, 7.9 (B)
H2'	5.21, dd, 8.1, 9.5	5.13, dd, 3.6, 10.3	5.29, t, 9.8	6.33, t, 9.2	5.49, t, 9.3	5.30, t, 7.9 (A) 5.26, t, 8.1 (B)
H3'	5.50, t, 9.5	5.70, dd, 9.6, 10.2	5.60, t, 9.5	5.50, t, 9.4	5.62, t, 9.5	5.51, t, 9.1 (A) 5.45, t, 9.3 (B)
H4'	5.20, t, 9.7	5.22, dd, 9.6, 10.2	5.42, t, 9.4	5.23, t, 9.9	5.32, t, 9.8	5.28, t, 9.3 (A) 5.24, t, 9.5 (B)
H5'	4.47, d, 9.8	4.46, dd, 10.2, 0.5	4.62, d, 10.0	4.49, d, 10.0	4.67, d, 10.1	4.66, d, 9.4 (A) 4.57, d, 9.6 (B)
$COOCH_3$	3.67, s	3.67, s	3.70, s	3.70, s	3.71, s	3.69, s, 3.67, s
CH_3COO	2.01, s 1.99, s 1.98, s	2.02, s 2.015, s 1.97, s	2.01, s 1.99, s 1.91, s	2.00, s 1.98, s 1.90, s	2.01, s 2.00, s 1.95, s	2.03, s, 2.01, s 2.003, s, 1.997, s 1.98, s, 1.975, s

[a] Spectra were recorded in CD_3COCD_3. ∂ are expressed in ppm relative to TMS. J_{H-H} or $_{H-F}$ are expressed in Hz. The signals of the protons H1' to H5' of the sugar moiety were assigned from H,H COSY.

[b] ^4J long-range couplings between H1' and H5', H1' and H3' and H3' and H5' were detected in H,H COSY spectra. Only $^4J_{H1'-H5'}$ was detected in the one-dimensional ^{1}H NMR spectra.

[c] H1' is long-ranging coupled through five bonds with ^{19}F nucleus.

[d] The two sugar moieties are denoted as A and B. They are bound to the pyrimidine ring by oxygens linked to C2 or C4 of FU. Their respective position was not established.

[e] J_{H-F} coupling constant.

[f] (b) = broad.

TABLE 2. ^{13}C NMR characteristics of protected glucuronides of FC and FU.

	∂, multiplicity, J[a]					
compound	O2-ß-FC 4a	O2-α-FC 6a	N1-ß-FC 5a	N3-ß-FC 7a	N1-ß-FU 8a	di-O2,O4-ß-FU[b] 9a
C2	159.3, d, 1.8	159.15, d, 1.7	154.1, s	148.9, s	149.8, s	158.1, s
C4	156.5, d, 13.7	156.5, d, 13.8	159.1, d, 13.4	154.3, d, 30.8	157.1, d, 27.6	158.2, d, 9.3
C5	144.1, d, 247.9	144.1, d, 247.9	137.5, d, 242.6	138.8, d, 221.1	141.6, d, 234.0	144.0, d, 256.2
C6	140.9, d, 20.8	141.2, d, 20.7	126.6, d, 32.4	118.3, d, 35.8	125.3, d, 35.2	147.0, d, 21.2
C1'	95.2	92.2	81.7	79.9	81.0	95.7 (B), 94.5 (A)
C2'	71.4	70.4	69.9	69.1	70.0	71.1 (B), 70.8 (A)
C3'	72.7	69.8	72.7	73.9	72.4	72.4 (B), 71.8 (A)
C4'	70.3	70.2	70.6	70.0	69.5	70.0 (B), 69.6 (A)
C5'	73.2	70.6	74.7	75.0	74.6	73.1 (2C, A and B)
C6'	167.9	168.1	167.7	167.8	167.4	167.65, 167.6
CH_3COO	170.2	170.4	170.0 (2C)	170.2	169.9 (2C)	170.2, 170.0
*	169.9	170.2		170.0	169.85	169.82, 169.79
	169.6	169.9	169.9	169.8		169.5
$COOCH_3$ *	52.9	52.9	52.9	52.8	52.9	53.0, 52.9
CH_3COO	20.5 (2C)	20.5	20.4 (2C)	20.5	20.35	20.43, 20.41 (2C)
*		20.4		20.45 (2C)	20.3	20.37 (2C), 20.33
	20.4	20.38	20.3		20.2	

[a] Spectra were recorded in CD_3COCD_3. ∂ are expressed in ppm relative to TMS. J_{C-F} are expressed in Hz. The signals of the carbons C1' to C5' of the sugar moiety were assigned from H,C COSY.

[b] The two sugar moieties are denoted as A and B. They are bound to the pyrimidine ring by oxygens linked to C2 or C4 of FU. Their respective position was not established.

Compound 5a was synthesized specifically by reacting the bromosugar 3 according to the method described by Vorbruggen and Bennua[7].

In the DCI/NH_3 mass spectrum, the signals at m/z 446 and 463 represent the molecular ions $[MH]^+$ and $[MNH_4]^+$ expected for the protected glucuronide 5a. The signals at m/z 317 and 130 were attributed to the pyronium ion and protonated FC respectively.

The nature of the linkage between the cytosine ring and the glucuronate moiety was established from the ^{13}C and ^1H NMR data. The C1' ∂ at 81.7 ppm is characteristic of a C1'-N bond (TABLE 2), and the coupling constant $^3J_{H1'-H2'}$ of 9.4 Hz indicates an axial-axial coupling, i.e. a ß configuration of the sugar (TABLE 1). The ^{19}F nucleus resonates as a doublet of doublet (dd) due to coupling between H6 of the cytosine ring (J = 6.9 Hz) and H1' of the sugar (J = 1.8 Hz). The same coupling constants were determined on the ^1H NMR spectrum from H6 and H1' signals (TABLE 1). The $^5J_{F-H1'}$ coupling is characteristic of derivatives of FU or FC substituted by a sugar on nitrogen 1[11].

The structure of the N1-ß-glucuronide of FC 5b formed by deprotection of compound 5a was established from the negative ion FAB mass spectrum (molecular ions $[M-H]^-$ and $[M-H+Na]^-$ (due to the sodiated medium) at m/z 304 and 326 respectively) and the ^{19}F, ^1H and ^{13}C NMR spectra. The C1' ∂ at 86.4 ppm (TABLE 4) is characteristic of a C-N linkage between cytosine and glucuronic acid, the $^3J_{H1'-H2'}$ of 7.3 Hz (TABLE 3) indicates a ß configuration of this linkage, while the existence of $^5J_{F-H1'}$ (TABLE 3) is indicative of a 5-fluoropyrimidine substituted by a sugar on N1.

Non specific synthesis of FC glucuronides

Condensation of unprotected and unactivated FC with the bromosugar 3 according to Wagner and Mahrwald[12,13] afforded four fluorinated compounds. Analysis of the reaction mixture by ^{19}F NMR showed the presence of four derivatives of FC resonating at -87.5, -87.6, -88.5 and -89.7 ppm (in CH_2Cl_2) in the following proportions 38%, 36%, 7% and 19%. On thin layer chromatography (TLC) (silica, elution with $CHCl_3$:CH_3OH, 92:8) they had Rf of 0.6, 0.7, 0.3 and 0.2 respectively, and they were thus separated on a silica column. Mass spectrometry and ^1H and ^{13}C NMR showed that the derivatives resonating at -87.5 ppm (Rf = 0.6) and -89.7 ppm (Rf = 0.2) corresponded to the O2-ß and N1-ß protected glucuronides of FC, 4a and 5a respectively. The two other compounds

TABLE 3. ^{1}H NMR characteristics of FC and FU glucuronides.

	∂, multiplicity, J[a]			
compound	O2-ß-FC 4b	O2-α-FC 6b	N1-ß-FC 5b	N1-ß-FU 8b
H6	7.82, d, 3.2[b]	7.85, d, 3.3[b]	7.91, d, 6.3[b]	8.03, d, 6.3[b]
H1'	5.61, d, 6.8	6.20, d, 3.7	5.62, dt, 7.3, 1.7, 1.7[c]	5.65, dd, 8.9, 1.7[d]
H2'	3.57-3.51[e], m	3.77, dd, 3.7, 9.8	3.69-3.61[f], m	3.81-3.54[f], m
H3'		3.88, t, 9.5		
H4'		3.55, dd, 9.1, 10.1	3.60-3.51, m	
H5'	3.82, d, 8.5	3.98, d, 10.1	3.87, d, 9.7	3.93, d, 9.6

[a] Spectra were recorded in D_2O. ∂ are expressed in ppm relative to TMPS. J_{H-H} or $H-F$ are expressed in Hz. The signals of the protons H1' to H5' of the sugar moiety were assigned from H,H COSY.

[b] J_{H6-F} coupling constant.

[c] $^3J_{H1'-H2'}$ = 7.3 Hz, $^5J_{H1'-F}$ = 1.7 Hz, $^5J_{H1'-H4'}$ = 1.7 Hz; this last interaction is also evident in the H,H COSY spectra.

[d] $^5J_{H1'-F}$.

[e] H,H COSY spectra demonstrated that H3' is more deshielded than H2', which is itself more deshielded than H4'.

[f] From H,H COSY spectra, it was established that H2' is more deshielded than H3', which is itself more deshielded than H4'.

were attributed from the behavior on TLC to an O-glucuronide (∂ = -87.6 ppm, Rf = 0.7) and an N-glucuronide (∂ = -88.5 ppm, Rf = 0.3).

- Determination of the structure of the O-substituted compound 6a and its deprotected homologue 6b

The DCI mass spectrum of the purified compound characterized by a ^{19}F NMR ∂ of -87.6 ppm and an Rf of 0.7 showed signals at m/z 446 $[MH]^+$, as expected for a protected glucuronide of FC, and at m/z 317 and 130 for the pyronium ion and protonated FC. In the ^{13}C NMR spectrum (TABLE 2), the ∂ of C1' at 92.2 ppm is characteristic of a C-O linkage. The high shielding of C1', C3' and C5' relative to the same carbon atoms in the protected O2-ß-glucuronide 4a (-2.6 to -3.0 ppm) indicates that the cytosine ring is in an axial position. Compound 6a is thus an O-glucuronide of configuration α. This configuration was supported by the weak $^3J_{H1'-H2'}$ coupling (3.6 Hz) in the ^{1}H NMR spectrum, indicative of an axial-equatorial coupling, and hence an α anomer[10] (TABLE 1). Compound 6a is thus methyl 2,3,4-Tri-O-acetyl-1-(4-amino-5-fluoro-pyrimidin-2-yl)-α-D-glucopyranosiduronate or the protected O2-α-FC glucuronide.

TABLE 4. ^{13}C NMR characteristics of FC and FU glucuronides.

	∂, multiplicity, J[a]			
compound	O2-ß-FC 4b	O2-α-FC 6b	N1-ß-FC 5b	N1-ß-FU 8b
C2	161.2, s	161.0, s	159.1, s	153.7, s
C4	158.3, d, 14.6	158.6, d, 15.0	161.3, d, 14.5	162.5, d, 25.6
C5	145.8, d, 247.4	145.6, d, 247.2	140.6, d, 244.5	143.8, d, 236.0
C6	142.4, d, 21.9	142.3, d, 21.7	128.9, d, 32.5	128.3, d, 34.6
C1'	99.6	97.1	86.4	85.4
C2'	75.0	73.1	74.2	73.7
C3'	78.1	75.3	78.7	78.4
C4'	74.4	74.5	74.3	74.0
C5'	79.2	76.2	81.4	81.2
C6'	178.1	178.6	178.1	177.8

[a] Spectra were recorded in D_2O. ∂ are expressed in ppm relative to TMPS. J_{C-F} are expressed in Hz. The signals of the carbons C1' to C5' of the sugar moiety were assigned from H,C COSY.

The structure of the O2-α-FC glucuronide 6b resulting from careful removal of acetylated and methylated protecting groups was determined from the mass spectra and the NMR data: molecular ion at m/z 304 $[M-H]^-$ in the negative ion FAB mass spectrum, C1' ∂ = 97.1 ppm characteristic of a C-O linkage between pyrimidine and sugar moieties, $^3J_{H1'-H2'}$ = 3.7 Hz characteristic of an α configuration for the C-O linkage (TABLES 3,4).

- Determination of the structure of the N-substituted compound 7a

The FAB mass spectrum in positive mode of the purified compound with a ^{19}F NMR ∂ of -88.5 ppm and an Rf of 0.3 showed the molecular ion $[MH]^+$ at m/z 446 characteristic of a protected glucuronide. The daughter ion spectrum of this ion afforded ions at m/z 386 (loss of one acetic acid molecule), 317 (pyronium ion), 257 (pyronium ion - one acetic acid molecule), 155 (pyronium ion - two acetic acid molecules - one ketene molecule) and 130 (protonated FC)[9,10].

The ^1H NMR spectrum (TABLE 1) showed that it was a ß anomer ($^3J_{H1'-H2'}$ = 9.4 Hz), while the ^{13}C NMR spectrum (TABLE 2) showed that it was an N-glucuronide (C1' ∂ = 79.9 ppm). Compound 7a is thus an N-glucuronide in the ß configuration, either N3-ß- or N7-ß-glucuronide. In the case of substitution on the exocyclic nitrogen, the electronic environment of H1' is comparable to that of the H1' proton in the protected O2-ß-glucuronide 4a, except for the presence of nitrogen instead of oxygen. Due to the

TABLE 5. UV absorption spectra for some cytosine derivatives.

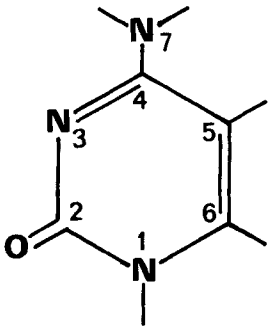
	λ_{max} (nm) neutral pH[a]	acid pH[b]	$\Delta\lambda$ (nm) neutral→cation	reference
cytosine	267	276	+9	14
5-fluorocytosine	275	281	+6	14
	276	278	+2	this work
1-methylcytosine	274	283	+9	14
3-methylcytosine	294	274	-20	14
7,7-dimethylcytosine	274	282	+8	14
5-fluorocytidine	281	290	+9	14
	280	292	+12	this work
N1-ß-FC 5a	276	284	+8	this work
N3-ß-FC 7a	324	296	-28	this work

[a] pH = 7.0 except for 3-methylcytosine (pH = 12.0).
[b] pH = 1.0 except for 3-methylcytosine (pH = 4.0).

difference in electronegativity between N and O, the H1' signal in 7a should be more shielded than in 4a. The observation of a downfield shift of 0.44 ppm for the H1' signal in 7a relative to 4a shows that 7a is not the N7-ß-glucuronide.

The N3-ß-glucuronide structure was confirmed from measurements of the UV spectra of FC, 5-fluorocytidine and the two N-ß-glucuronides 5a and 7a at different pH. The neutral species of cytosine, 5-halogenocytosines, 7,7-dimethylcytosine as well as their 1-methyl and 1-ß-D-ribofuranosyl derivatives in aqueous solution can be represented by structure I. Their cations are formed by protonation on N3[12,13]. When passing from neutral to cationic form, all these compounds exhibit a bathochromic shift of the position of the maximum absorption wavelength with a $\Delta\lambda_{max}$ of $\approx$ 9 nm[14] (TABLE 5). 3-methylcytosine and 6-halogenocytosines exist predominantly in aqueous solution in the 4-amino-2-oxo structure II[14-16]. Protonation of these compounds, which occurs on N1, is manifested by a hypsochromic shift of their maxima of 20 nm[14] (TABLE 5). Protonation of compound 7a induced a hypsochromic shift of 28 nm (TABLE 5). If compound 7a was the N7-glucuronide, its protonation would have given rise to a bathochromic shift of its absorption peak. Therefore 7a must be methyl 2,3,4-Tri-O-acetyl-1-deoxy-1-(5-fluoro-

cytosin-3-yl)-ß-D-glucopyranuronate. We wanted to check that protonation of com - pounds with a type I structure (FC, 5-fluorocytidine, N1-ß-FC 5a) did in fact show a bathochromic shift of their absorption peaks as reported by Wempen and Fox[14b]. The results shown in TABLE 5 show a bathochromic shift of their λ_{max} of 2 to 12 nm.



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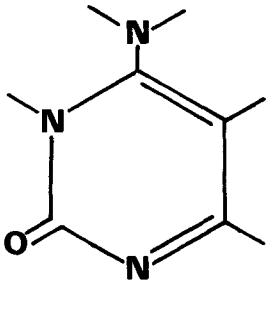


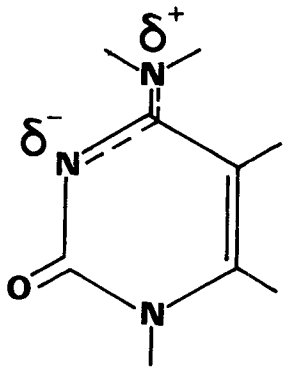
II

Deprotection of glucuronide 7a led to a mixture of five fluorinated compounds of which only fluoride anion and the O2-ß-FC glucuronide 4b could be identified.

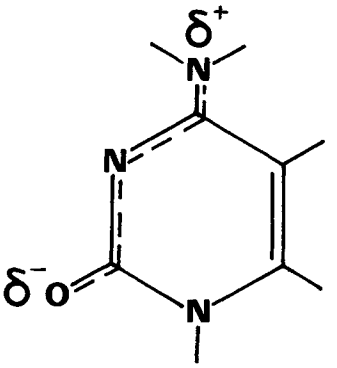
Conclusion

Of the four potential nucleophilic sites of FC, the oxygen on carbon 2 is most reactive towards the bromosugar 3. The O-glucuronides make up 74% of the products versus 19% from those derived from attack on nitrogen 1 and 7% from attack on nitrogen 3. This is in complete agreement with the theoretical study of Pullman and Armbruster[17] who compared the nucleophilicity of the N3 and C2=O positions. The preferred site of protonation is nitrogen 3, whereas for more bulky electrophiles ($C_2H_5^+$), the oxygen of the C2=O site is more accessible.

The absence of functional attack at the exocyclic amino group in our experiments can be accounted for by the partial double bond character of the C-N extracyclic bond due to resonance structures of types III and IV. Rotational restriction of the exocyclic amino group has been predicted from different computational methods, and has been observed by NMR on 7-methyl- and 7,7-dimethylcytosine derivatives[15].



III



IV

The ^{19}F NMR analysis of human urine samples spiked with the synthesized deprotected FC glucuronides showed that the glucuronide formed in the metabolism of FC is the O2-ß-FC glucuronide 4b.

Synthesis of FU glucuronides

Synthesis of the various O-monoglucuronides of FU has been reported by researchers of the Sankyo Co.[18-20], and that of the N-mono- and diglucuronides of FU by Mahrwald and Wagner[12]. However, the spectroscopic characteristics of these compounds (mass or ^{19}F, ^{1}H, ^{13}C NMR) so far have not been described. Furthermore, although a general scheme for synthesis of all the glucuronides of FU was reported in 1977[18], no experimental details for the preparation of the di-O-glucuronide of FU are available.

Synthesis of methyl 2,3,4-Tri-O-acetyl-1-deoxy-1-(5-fluorouracil-1-yl)-ß-D-glucopyranuronate (protected N1-ß-FU glucuronide) 8a and 1-deoxy-1-(5-fluorouracil-1-yl)-ß-D-glucopyranuronic acid (N1-ß-FU glucuronide) 8b

The protected N1-ß-FU glucuronide 8a was synthesized by reacting the bromosugar 3 with persilylated FU in the presence of $SnCl_4$[7,12].

The structure of the obtained compound 8a was determined by mass and NMR spectroscopy. DCI with ammonia as carrier gas showed molecular ions at m/z 447 $[MH]^+$ and m/z 464 $[MNH_4]^+$. The chemical shift of C1' (81.0 ppm) in the ^{13}C NMR spectrum (TABLE 2) demonstrates the existence of a C-N linkage between the sugar and the FU

moiety. The high value of $^{3}J_{H1'-H2'}$ (9.3 Hz) is indicative of a ß configuration for the C-N linkage, while the $^{5}J_{H1'-F}$ coupling (1.7 Hz on ^{1}H NMR spectrum or 1.5 Hz on ^{19}F NMR spectrum) shows that the nitrogen 1 of the pyrimidine ring is substituted[11].

Deprotection afforded, after purification by HPLC, 1-deoxy-1-(5-fluorouracil-1-yl)-ß-D-glucopyranuronic acid 8b, which was characterized by its negative ion mass spectrum (molecular ion at m/z = 305), and ^{1}H and ^{13}C NMR spectra (C1' ∂ = 85.4 ppm: N-glucuronic acid, $^{3}J_{H1'-H2'}$ = 8.9 Hz: ß linkage between the sugar and the pyrimidine moiety, $^{5}J_{F-H1'}$ = 1.7 Hz: N1-glucuronic acid) (TABLES 3,4).

Synthesis of dimethyl 2,2',3,3',4,4'-Hexa-O-acetyl-1,1'-(5-fluoro-pyrimidin-2,4-diyl)-ß-D-glucopyranosiduronate (protected O2,O4 bis-ß-FU glucuronide) 9a

This compound was obtained by condensation of the silver salt of FU with acetobromoglucose 3. The DCI/NH_3 mass spectrum showed molecular ion $[MH_2]^{2+}$ at m/z 764 and the ion $[MHNH_4]^{2+}$ at m/z 781. ^{13}C and ^{1}H NMR data demonstrated the presence of two C-O linkages between FU and the sugar moiety (C1' ∂ = 95.7 and 94.5 ppm) with a ß configuration ($^{3}J_{H1'-H2'}$ = 7.5 and 7.9 Hz) (TABLES 1,2). Deprotection of 9a led to extensive degradation.

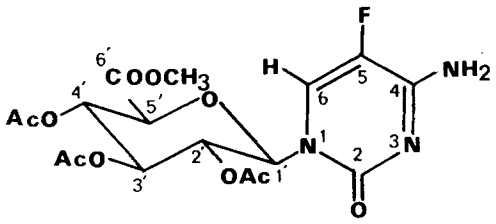
Syn/anti preferred orientation about N-glycosidic bond

The long-range five-bond spin-spin coupling interactions between H5 or F5 and H1' detected in pyrimidine nucleosides were rationalized by a predominantly anti conformation for each nucleoside (5,6 double bond endo or C2=O exo to the sugar ring). Indeed, this conformation places the H5 or F5 and H1' in the zig-zag or "W" configuration, which favors long-range couplings[11]. In orotidine, the anti conformation is not favored since it means that the 6-substituent (namely the bulky carboxyl group) lies above the sugar. This sterically unfavorable interaction suggested that the syn conformation (2-keto oxygen above the sugar ring) is preferred for this compound, as demonstrated from ^{1}H NMR chemical shifts and coupling constants of the ribose ring[21]. In the syn conformation, H5 and H1' are not connected by an extended zig-zag path, and therefore no five-bond coupling is detectable[22].

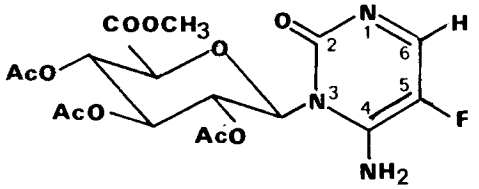
As a consequence, a predominantly anti conformation V was indicated by the five-bond coupling between F5 and H1' in protected and deprotected N1-FC and FU glucuro-

nides (5a, 5b, 8a, 8b). The fact that this coupling exceeded 1.5 Hz (^{1}H NMR spectra) confirmed the ß configuration of the linkage between the sugar and the pyrimidine ring since the magnitude of this long-range coupling has been shown to be > 1.5 Hz for ß compounds and < 1.5 Hz for α compounds[11].

The protected N3-ß-FC glucuronide 7a did not exhibit any long-range coupling between F5 and H1', and is thus most likely to be in the syn conformation VI. This conclusion is logical as placement of the 2-keto oxygen over the sugar ring (syn conformation) is sterically more favorable than that of the 4-amino group (anti conformation).



V, anti conformation
N1-ß-FC glucuronide 5a



VI, syn conformation
N3-ß-FC glucuronide 7a

EXPERIMENTAL SECTION

General methods

^{1}H, ^{19}F and ^{13}C NMR spectra were recorded at 300, 282.4 and 75.4 MHz respectively on a Bruker AM 300 WB spectrometer. Chemical shifts are reported in parts per million (ppm) relative to (i) tetramethylsilane (TMS) (or 3-(trimethylsilyl)-propane sulfonic acid sodium salt (TMPS) for compounds in aqueous solution) as internal standards for the ^{1}H and ^{13}C NMR spectra, and (ii) trifluoroacetic acid (CF_3COOH) in 5% (w/v) aqueous solution as an external standard for the ^{19}F NMR spectra. Notations used are s: singlet, d: doublet, t: triplet, dd: doublet of doublet, dt: doublet of triplet, m: multiplet and b: broad. Total assignments of the ^{1}H and ^{13}C signals were obtained from analysis of two-dimensional homonuclear (HH) or heteronuclear (HC) correlated NMR spectra (2D H,H COSY and 2D H,C COSY). DCI mass spectra were obtained with a Nermag R10A spectrometer with ammonia as carrier gas, and FAB mass spectra in positive or negative mode

with a VG ZAB HS spectrometer. The UV spectra were recorded on a Hewlett-Packard 8452A spectrometer. Melting points (mp) were taken on a Reichert apparatus and were uncorrected. The HPLC system consisted of a Waters model with a photodiode array detector. TLC used Merck silica gel plates GF_{254}.

All the compounds synthesized were characterized by NMR (^{19}F, ^{1}H, ^{13}C, 2D H,H COSY and 2D H,C COSY) and mass spectrometry. In some cases, UV spectra were also recorded.

Syntheses

Methyl 1,2,3,4-Tetra-O-acetyl-ß-D-glucopyranuronate 2

Compound 2 was prepared according to the procedure of Bollenback et al.[23]. Yield: 60%; mp: 178°C (lit.[23]: 176.5-178°C). ^{1}H NMR ($CDCl_3$): 5.78 (H1, d, 7.7 Hz characteristic of a ß anomer form), 5.14 (H2, dd, 7.9 Hz with H1 and 8.8 Hz with H3), 5.32 (H3, AB system with H4 J = 9.1 Hz, J = 8.8 Hz with H2), 5.24 (H4, AB system with H3 J = 9.1 Hz, J = 9.4 Hz with H5), 4.20 (H5, d, 9.4 Hz), 3.75 (CH_3OCO, s), 2.12 (CH_3COO, s), 2.04 (2 x CH_3COO, s), 2.03 (CH_3COO, s). Assignments of H2, H3 and H4 were established by irradiation of H1 and H5 resonances. ^{13}C NMR ($CDCl_3$): 169.9, 169.4, 169.2, 168.8 (4 x $CH_3\underline{C}OO$), 166.8 ($CH_3O\underline{C}O$), 91.4 (C1), 70.2 (C2), 71.9 (C3), 68.9 (C4), 73.0 (C5), 53.0 ($\underline{C}H_3OCO$), 20.8 ($\underline{C}H_3COO$), 20.6 (2 x $\underline{C}H_3COO$), 20.5 ($\underline{C}H_3COO$). Assignments of C1-C5 were obtained from 2D H,C COSY.

Methyl 2,3,4-Tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate 3

Compound 3 was prepared according to the procedure of Bollenback et al.[23]. Yield: 65%; mp: 107°C (lit.[23]: 106-107°C). ^{1}H NMR ($CDCl_3$): 6.61 (H1, d, 4.1 Hz characteristic of an α anomer form), 4.82 (H2, dd, 4.1 Hz with H1 and 10.0 Hz with H3), 5.58 (H3, t, 9.7 Hz), 5.20 (H4, dd, 9.6 Hz with H3 and 10.0 Hz with H5), 4.54 (H5, d, 10.3 Hz), 3.78 (CH_3OCO, s), 2.11 (s), 2.07 (s) and 2.06 (s) (each s corresponds to one CH_3COO group). ^{13}C NMR ($CDCl_3$): 169.6 (2 x $CH_3\underline{C}OO$), 169.4 ($CH_3\underline{C}OO$), 166.6 ($CH_3O\underline{C}O$), 85.4 (C1), 72.0, 70.3, 69.3, 68.5 (C2, C3, C4, C5), 53.1 ($\underline{C}H_3O$), 20.6 (2 x $\underline{C}H_3COO$), 20.4 ($\underline{C}H_3COO$).

FC silver salt

A solution of silver nitrate (0.66 g) in 5 ml of water was added to a solution of FC (0.58 g) in 50 ml of hot dilute (2%) aqueous ammonia[3a]. After stirring for 2 h in a steam

bath, the solution was cooled overnight at 4°C. The crystals obtained were isolated by filtration, washed with ether then dried. Yield: 78%.

Methyl 2,3,4-Tri-O-acetyl-1-(4-amino-5-fluoro-pyrimidin-2-yl)-ß-D-glucopyranosiduronate (protected O2-ß-FC glucuronide) 4a (specific synthesis)

1.6 g (6.8 mmoles) of the silver salt of FC and 2.6 g (6.6 mmoles) of compound 3 were added under an inert atmosphere (stream of argon) to 40 ml of anhydrous xylene under reflux. The mixture was stirred and refluxed for 20 min. The solution was filtered and the residue (AgBr) washed with $CHCl_3$. The filtrate was poured into 300 ml of hexane. The precipitate obtained was filtered off, washed with hexane and dried. Crystallization from hexane afforded compound 4a in 58% yield. Mp: 86°C. Microanalysis: calculated: C: 45.84%, H: 4.49%, O: 35.95%, N: 9.40%; found: C: 44.93%, H: 4.24%, O: 35.79%, N: 9.36%. ^{19}F NMR ($CDCl_3$): -87.7 (d, 2.7 Hz). Mass and ^1H and ^{13}C NMR characteristics are reported in the text.

Methyl 2,3,4-Tri-O-acetyl-1-deoxy-1-(5-fluorocytosin-1-yl)-ß-D-glucopyranuronate (protected N1-ß-FC glucuronide) 5a (specific synthesis)

0.5 g (3.87 mmoles) of FC, 1.45 g (3.88 mmoles) of bromosugar 3 and 3.14 g (9.3 mmoles) of potassium nonaflate ($C_4F_9SO_3K$) were refluxed under argon in 60 ml anhydrous acetonitrile with 0.65 ml (3.05 mmoles) of hexamethyldisilazane (HMDS) and 0.86 ml (6.8 mmoles) of trimethylchlorosilane (TMCS). To the orange turbid solution obtained after 21 h reflux, 100 ml of $CHCl_3$ were added. The mixture was extracted with saturated bicarbonate aqueous solution. After reextracting the aqueous phase with 50 ml of CH_2Cl_2, the combined organic phases were washed with saturated aqueous NaCl, dried (Na_2SO_4) and evaporated. Crystallization from hexane afforded compound 5a in 20% yield. Mp: 134-135°C. Microanalysis: calculated: C: 45.84%, H: 4.49%, O: 35.95%, N: 9.40%; found: C: 44.77%, H: 4.51%, O: 35.83%, N: 9.32%. ^{19}F NMR ($CDCl_3$): -90.0 (dd, 6.9 Hz with H6 and 1.8 Hz with H1'). Mass and ^1H and ^{13}C NMR characteristics are reported in the text.

Non specific synthesis of FC protected glucuronides

0.6 ml (5.28 mmoles) of anhydrous stannic chloride ($SnCl_4$) were added to a solution of 0.5 g (3.87 mmoles) of FC and 1.3 g (3.38 mmoles) of bromosugar 3 in 10 ml of anhydrous acetonitrile. The reaction mixture was stirred at room temperature for 2.5 h. The solvent was then evaporated under reduced pressure and the residue dissolved in 50

ml of CH_2Cl_2. The solution thus obtained was extracted with saturated aqueous bicarbonate solution, the organic phase was filtered on Celite 545, dried (Na_2SO_4) and evaporated. The residue was column-chromatographed on silicagel (Kieselgel 60, 0.063-0.100 mm), and eluted with $CHCl_3$:CH_3OH (92:8). The fractions were collected, and the separation was monitored by TLC using $CHCl_3$:CH_3OH (92:8) as the developing solvent. Four compounds were isolated. Apart from the protected O2-ß-glucuronide 4a (Rf = 0.6) and the protected N1-ß-glucuronide 5a (Rf = 0.2), two other compounds were purified. From their values of Rf and their mass, NMR and UV characteristics (see text), they were identified as methyl 2,3,4-Tri-O-acetyl-1-(4-amino-5-fluoro-pyrimidin-2-yl)-α-D-glucopyranosiduronate 6a (Rf = 0.7) and methyl 2,3,4-Tri-O-acetyl-1-deoxy-1-(5-fluorocytosin-3-yl)-ß-D-glucopyranuronate 7a (Rf = 0.3). The net yields after chromatography were 30% for the O2-ß derivative 4a, 10% for the N1-ß derivative 5a, 5% for the O2-α derivative 6a (mp: 78-79°C; microanalysis: calculated: C: 45.84%, H: 4.49%, O: 35.95%, N: 9.40%; found: C: 44.87%, H: 4.44%, O: 35.81%, N: 9.31%; ^{19}F NMR ($CDCl_3$): -87.65 (d, 2.7 Hz)) and 2% for the N3-ß derivative 7a (microanalysis: calculated: C: 45.84%, H: 4.49%, O: 35.95%, N: 9.40%; found: C: 45.18%, H: 4.50%, O: 35.90%, N: 9.32%; ^{19}F NMR ($CDCl_3$): -87.9 (broad signal: the coupling between F and H6 was undetected but observed in the ^1H NMR spectrum (TABLE 1)).

Methyl 2,3,4-Tri-O-acetyl-1-deoxy-1-(5-fluorouracil-1-yl)-ß-D-glucopyranuronate (protected N1-ß-FU glucuronide) 8a (specific synthesis)

0.47 ml (2.25 mmoles) of HMDS, 0.29 ml (2.25 mmoles) of TMCS and 2.40 ml (20.4 mmoles) of $SnCl_4$ were added to a solution of 0.35 g (2.69 mmoles) of FU and 1.00 g (2.67 mmoles) of bromosugar 3 in 60 ml anhydrous acetonitrile under argon. After stirring for 10 h at room temperature, 100 ml of CH_2Cl_2 were added. Work up as described for the protected N1-ß-FC glucuronide 5a afforded a crude orange product which was column-chromatographed on silicagel as described above for the non-specific synthesis of FC glucuronides. Yield: 20%; mp: 225°C (lit.[12]: 226-227°C). ^{19}F NMR ($CDCl_3$): -86.1 (apparent td, 5.7 Hz with H6, 1.5 Hz with H1' and 4.9 Hz with H3-N; these three F-H couplings were also observed in the corresponding ^1H NMR spectrum). Mass and ^1H and ^{13}C NMR characteristics are reported in the text.

Dimethyl 2,2',3,3',4,4'-Hexa-O-acetyl-1,1'-(5-fluoro-pyrimidin-2,4-diyl)-ß-D-glucopyranosiduronate (protected O2,O4 bis-ß-FU glucuronide) 9a (specific synthesis)

6.8 mmoles of the silver salt of FU (obtained in 66% yield from FU using the procedure described above for FC silver salt) and 14 mmoles of bromosugar 3 were added under argon to 40 ml of boiling anhydrous xylene. The mixture was stirred and refluxed for 20 min. Work up as for the protected O2-ß-FC glucuronide 4a led to a orange syrup, which was column-chromatographed on silicagel as described in the non-specific synthesis of the FC-glucuronides. Yield: 25%; mp: 214°C. ^{19}F NMR ($CDCl_3$): -84.2 (d, 1.6 Hz). Mass and ^{1}H and ^{13}C NMR characteristics are reported in the text.

General method for synthesis of ß and α-D-glucuronic acids of FC or FU

The protecting acetyl and methyl groups were carefully removed from the glucuronides 4a-9a according to the procedure described in the Sankyo patent[20]. 0.27 mmoles of protected glucuronide were dissolved in 1.4 ml of anhydrous CH_3OH. After cooling the mixture in iced water, 0.16 ml of a 1 M solution of sodium methylate in methanol were added. The reaction mixture was stirred for 40 min at 0°C and then neutralized with 1.2 M HCl (0.13 ml). Solvent was evaporated under reduced pressure. The residue was dissolved in 4 ml of $CHCl_3$ and the solution obtained extracted with 4 ml of water. 5.85 ml of CH_3OH and 0.1 ml of aqueous ammonia (20%) were added to the aqueous phase. The solution was stirred at room temperature for 24 h, solvent was then evaporated, leaving a whitish solid material.

1-(4-amino-5-fluoro-pyrimidin-2-yl)-ß-D-glucopyranosiduronic acid (O2-ß-FC glucuronide) 4b (yield: 70%; ^{19}F NMR (H_2O): -86.5 (d, 3.1 Hz)) and 1-deoxy-1-(5-fluorocytosin-1-yl)-ß-D-glucopyranuronic acid (N1-ß-FC glucuronide) 5b (yield: 66%; microanalysis: calculated: C: 39.34%, H: 3.93%, O: 36.72%, N: 13.77%; found: C: 38.84%, H: 3.65%, O: 36.15%, N: 13.44%; ^{19}F NMR (H_2O): -88.5 (dd, 6.2 Hz with H6 and 1.1 Hz with H1')) were sufficiently pure for mass spectrometry and NMR spectroscopy.

1-(4-amino-5-fluoro-pyrimidin-2-yl)-α-D-glucopyranosiduronic acid (O2-α-FC glucuronide) 6b and 1-deoxy-1-(5-fluorouracil-1-yl)-ß-D-glucopyranuronic acid (N1-ß-FU glucuronide) 8b were purified by HPLC using a Lichrosorb RP-18 (7 μm) analytical column. The mobile phase consisted of 95% H_2O-5% CH_3OH. The flow rate was set at 1 ml/min and the UV absorbance of the column effluent was monitored at 280 nm. The products were characterized by mass spectrometry and NMR. ^{19}F NMR (H_2O): 6b: -86.8

(d, 3.2 Hz); 8b: -89.2 (d, 5.6 Hz; the coupling between F and H1' was undetected but observed in the ^{1}H NMR spectrum (1.7 Hz, TABLE 3)).

Deprotection of methyl 2,3,4-Tri-O-acetyl-1-deoxy-1-(5-fluorocytosin-3-yl)-ß-D-glucopyranuronate 7a and dimethyl 2,2',3,3',4,4'-Hexa-O-acetyl-1,1'-(5-fluoropyrimidin-2,4-diyl)-ß-D-glucopyranosiduronate 9a afforded several products. We were unable to obtain pure compounds by HPLC.

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